# Gene Array Profiling and Immunomodulation Studies Define a Cell-Mediated Immune Response Underlying the Pathogenesis of Alopecia Areata in a Mouse Model and Humans

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**Alopecia areata is a suspected autoimmune hair loss disease. In a rodent model, alopecia areata can be induced in normal haired C3H/HeJ mice by transfer of skin grafts from mice with spontaneous alopecia areata. At weeks 2, 4, 6, and 10 after surgery, grafted mice were euthanized, skin collected and processed for histology, and RNA extracted. Age-matched sham-grafted mice, and mice with and without spontaneous alopecia areata, were similarly processed. For comparison, skin biopsies from alopecia areata and androgenetic alopecia affected humans were also collected. Skin mRNA processed to cDNA was analyzed using Affymetrix mouse 11K and human 6800 gene chip® array technology. Microarray results indicated 42 known genes upregulated or downregulated during onset of mouse alopecia areata consistent with an inflammatory cell-mediated disease pathogenesis involving antigen presentation, costimulation, and a T helper 1 lymphocyte response. In contrast, 114 genes, many regulating immunoglobulin response, were altered late in disease development. In alopecia areata affected humans, 95 genes were significantly modulated. As confirmation of microarray analysis results, lymph node and spleen cells from alopecia areata affected mice injected into normal haired littermates transferred the alopecia areata phenotype. Alopecia areata onset could be inhibited in skin-grafted mice by modulation with B7.1- and B7.2-specific monoclonal antibodies. In addition, depletion of $CD4^+$ $CD8^+$ expressing cells in chronic alopecia areata affected mice using monoclonal antibodies permitted hair regrowth. The results consistently demonstrated the importance of an immune cell-mediated disease mechanism in alopecia areata pathogenesis and suggested targeting antigen-presenting cells and reactive lymphocytes may be effective in alopecia areata treatment.** ***Key words: autoimmune/B7.1/B7.2/CTLA-4-mIgG2am/lymphocyte costimulation/rodent model. J Invest Dermatol 119:392–402, 2002***

Alopecia areata (AA) in humans is a spontaneous reversible form of hair loss with a lifetime risk of 1.7% (Hordinsky, 1994; Safavi *et al*, 1995). The disease can affect any hair bearing region of the skin and is seen in men, women, and children. Hair loss can develop in distinct patches or, occasionally, diffuse areas most frequently on the scalp. In 7% of cases, alopecia may progress to total scalp hair loss (alopecia totalis, AT) or total body hair loss (alopecia universalis, AU) (Safavi *et al*, 1995). Current concepts of AA disease pathogenesis suggest AA is a nonscarring, inflammatory, cell-mediated disease. A perifollicular and intrafollicular mononuclear cell infiltrate of primarily $CD4^+$ and $CD8^+$ cells is closely associated with dystrophic anagen stage hair follicles (Perret *et al*, 1984; Ranki *et al*, 1984) and immunomodulatory agents have been used to induce hair regrowth (Gupta *et al*, 1990; Hoffmann *et al*, 1994). These and other circumstantial evidence suggest that AA may be an autoimmune disease of anagen stage hair follicles, although there is no direct evidence in support of this hypothesis (McElwee *et al*, 1999c).

A number of species, including several mouse strains, develop circumscribed hair loss associated with nonscarring inflammation of dystrophic anagen hair follicles (Sundberg *et al*, 1995; McElwee *et al*, 1998b, 1999a). Of these, the C3H/HeJ inbred mouse strain has been identified as a promising mouse model for AA (Sundberg *et al*, 1994a). The C3H/HeJ strain develops AA-like hair loss with a spontaneous expression rate of 20% in mice aged 18 mo or older. Breeding studies indicate that AA-like hair loss has a polygenic dominant or semidominant basis with partial penetrance (Sundberg *et al*, 1994a; McElwee *et al*, 2001). Whereas a low spontaneous expression rate is typical of a polygenic disease (Vyse and Todd, 1996; Morel *et al*, 1994), it limits the usefulness of any spontaneous mouse model in disease research.

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Abbreviations: AA, alopecia areata; APC, antigen-presenting cell; AT, alopecia totalis; AU, alopecia universalis; HLA, human leukocyte antigen.

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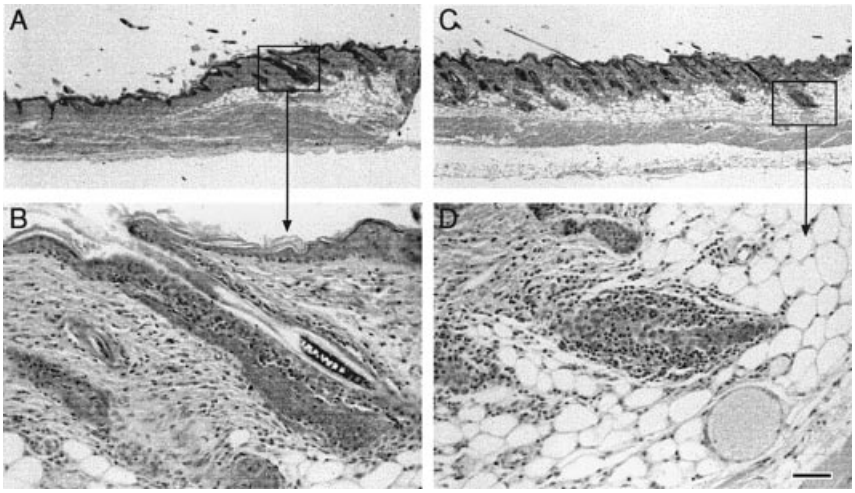


**Figure 1. Transfer of alopecia areata from affected to unaffected mice by skin grafting.** Ten weeks after receiving a full thickness skin graft from an histocompatible mouse with AA, the recipient developed AA adjacent to the graft site (*A*, *B*) and at distant site (*C*, *D*). Lymphocytes were present in and around anagen stage hair follicles. *Scale bar*: (*A*, *C*) 80 μm, (*B*, *D*) 20 μm.

Recently, a simple AA-induction technique was developed. Full thickness AA-affected skin grafts from spontaneous AA-affected C3H/HeJ mice can be used to induce hair loss in naïve, normal haired adult recipients (McElwee *et al*, 1998a). Typically, 8–10 wk after surgery, graft recipients will develop ventral and then dorsal hair loss. This technique is a suitable model to define the dynamics of AA after the disease activation event, in advance of overt hair loss, and during the initial stages of alopecia.

In order to examine the kinetics of cellular and molecular events leading to hair loss, the naturally occurring and skin graft induced AA mouse models were used. Gene expression patterns were determined in advance and during the initial stages of hair loss. These gene profiling data were compared with similar data derived from human patients with the severe forms of AA, AT, and AU. By using gene expression profiling, it was possible to determine the relative timing of important molecular events and compare these with those observed in various forms (stages) of human AA. Although gene array technology is most useful as a large-scale screening tool, several possible modes of disease induction and intervention in mouse AA-like disease development were identified based on gene array data analysis that were subsequently selected for further examination. Lymphocyte depletion in AA-affected mice or transfer of cells from affected to clinically normal mice revealed these were the primary effector cells. Agents specific for antagonizing lymphocyte costimulation pathways were used in the mouse skin graft model to determine whether interfering with these events was sufficient to prevent disease onset. These studies demonstrate (i) that the C3H/HeJ mouse model for AA is similar in many respects to the human clinical disease, and (ii) agents that block T cell activation through costimulation pathways are sufficient to prevent onset of disease in mice.

## MATERIALS AND METHODS

**Mice** Spontaneous AA-affected and AA-unaffected C3H/HeJ mice were obtained from the Jackson Laboratory (Bar Harbor, ME). All mice received autoclaved food pellets (NIH 31 modified with 6% fat, diet code 5K52, PMI, Richmond, IN) and acidified water *ad libitum*. All procedures were conducted with Institutional Animal Care and Use Committee (IACUC) approval. Normal haired mice were grafted with AA-affected skin using previously described techniques (McElwee *et al*, 1998a). Age-matched C3H/HeJ mice were killed by $CO_2$ asphyxiation at 2, 4, 6, and 10 wk after skin grafting. For each time point, four mice grafted with AA-affected skin and four sham-grafted control mice were studied. In addition, for gene expression analysis, three mice with spontaneous, chronic, extensive AA persisting for at least 6 mo were used as positive controls and three unaffected wild-type littermates were used as negative controls. Representative skin taken from the graft and immediately adjacent skin as well as distant sites of dorsal and ventral skin were fixed in Fekete's acid-alcohol formalin and processed routinely for histology (Relyea *et al*, 2000).

**Human AT patients and controls** Approval to perform a 4 mm biopsy with suitable anesthesia and precautions of human scalp skin was received from the Vanderbilt University Committee for the Protection of Human Subjects. Three subjects with histologically documented AA (AU: 30 y old female caucasian, AT: 44 y old female caucasian, and 43 y old male caucasian), no known hair or scalp skin problems (39 y old female caucasian, 52 y old female caucasian), and a patient with androgenetic alopecia (60 y old male caucasian) volunteered. A biopsy of a site of actively growing hair (chest) was also obtained from the volunteer with androgenetic alopecia to compare with his noninflamed androgenetic alopecia site. Evaluation of biopsy sites at the time of suture removal and subsequent follow-up found no complications. In the acute or subacute stage all AA patients had exclamation point hairs, a positive hair pull test, and histologically documented AA. At the time of the biopsies for this study, the patients had chronic, nonscarring AA with no obvious terminal hairs and no evidence of inflammation. No histologic exam of a portion of the specimens for DNA microarray was done. Four samples of normal breast skin, removed and discarded at the time of breast reduction surgery, were also tested.

**RNA purification and quantitative reverse transcriptase–polymerase chain reaction (reverse transcriptase–PCR) and Gene chip analysis** After being euthanized, mice were shaved with mechanical clippers and total, full thickness dorsal and ventral skin were dissected free. Underlying fascia and soft tissue were removed. RNA was prepared by cutting skin into small (2.0 cm) pieces and snap freezing them in liquid nitrogen. Frozen skin was homogenized in a Pyrex tube containing Tri-reagent system (5 ml per 2 $cm^2$ skin; Molecular Research Center, Cincinnati, OH) using a Polytron tissue homogenizer (Westbury, NY) for 1 min on ice. After RNA precipitation, RNA was purified using an RNeasy spin column system according to the manufacturer's instructions (Qiagen, Chatsworth, CA). Purified RNA was then resuspended in diethyl pyrocarbonate-treated water and stored at –80°C until further use. The same procedures were used for human skin.

Total RNA was then converted to cDNA by priming with an oligo(dT) primer that included a T7 RNA polymerase promoter site at the 5′ end (Lockhart *et al*, 1996). The cDNA was used directly in an *in vitro* transcription reaction in the presence of biotinylated nucleotides to produce labeled cRNA (anti-sense RNA), that was hybridized overnight to Gene chips (Affymetrix, San Jose, CA). The mouse 11K and human 6800 gene chips were used for these studies. After labeling with phycoerythrin–streptavidin, the fluorescence of bound RNA was quantitated by using a Gene chip Reader (a modified confocal microscope; Affymetrix) and analyzed as previously described (Wilson *et al*, 2000).

**Quantitative PCR** Samples were reverse transcribed for 30 min at 60°C and then subjected to 40 rounds of amplification for 15 s at 95°C and 1 min at 60°C using the ABI Prism 7700 sequence detection system as described by the manufacturer (Perkin Elmer, Wellesley, MA). Sequence-specific amplification was detected as an increased fluorescence

**Table I. Upregulated transcripts (⩾ 3-fold) in mice receiving AA affected skin grafts at 4–6 wk after grafting compared with no changes in those mice receiving normal, unaffected skin grafts (42 known genes, 16 expressed sequence tags)**

| GenBank accession no. | Common gene name | Official gene symbol/name/type |
|---|---|---|
| X69902_s_at | Integrin α6 | *Itga6*: integrin α6 (other) |
| X99807_s_at | Selenoprotein P | *Sepp1*: selenoprotein P, plasma, 1 (other) |
| Z11974_s_at | Mrc1 | *Mrc1:* mannose receptor, C type 1 (other) |
| L20315 | Mpeg1 | *Mpeg1:* macrophage expressed gene 1 (macro) |
| X00619 | Tcrb-V13 | *Tcrb-V13:* T cell receptor β, variable chain 13 (T cell) |
| X00958 | I-E (class II) | *H2-I-Eb* (I-E (β-b)) (macro/APC) |
| X03151_s_at | Thy 1 antigen | *Thy1*: thymus cell antigen 1, θ (T cell) |
| X03533_s_at | Tyrosine protein kinase p56-tck | *Lck*: lymphocyte protein tyrosine kinase (T cell) |
| X04648_s_at | FcR | *Fcgr2b*: Fc receptor, IgG, low-affinity Iib (macro/APC) |
| X14951_s_at | LFA-1/CD18 | *Itgb2*: integrin β2 (cd18) (T cell) |
| X16133_s_at | Serglycin | *Prg*: proteoglycan, secretory granule (other) |
| X16874_f_at | Complement C1q β | *C1qb*: complement component 1, q subcomponent, β polypeptide (general immune) |
| X53247_s_at | En-7 (T cell specific ras) | *Rac2*: RAS-related C3 botulinum substrate 2 (T cell) |
| X53526_s_at | BCM1/CD48 | *Cd48*: CD48 antigen (macro/APC) |
| X54511_f_at | mbh1 | *Capq*: capping protein (actin filament), gelsolin like (other) |
| X58861_s_at | Complement C1q α | *C1qa*: complement component 1, q subcomponent, α polypeptide (general immune) |
| X62743_s_at | Mb | *H2-DMb1* or *H2-DMb2*: histocompatibility 2, class II, locus Mb1 or Mb2 (mRNA) (macro/APC) |
| X66295_s_at | Complement C1q c-chain | *C1qc*: complem (general immune) |
| X67809_s_at | Mama (macrophage scavenger receptor) | *Ppicap*: peptidylprolyl isomerase C-associated protein (macro/APC) |
| X72862_s_at | β-3-adrenergic | *Adrb3*: adrenergic receptor, β 3 (other) |
| X75129_s_at | Xanthine dehydrogenase | *Xdh*: xanthine dehydrogenase (other) |
| X84797_s_at | HSP-1 (hematopoietic specific protein 1) | *Hcls1*: hematopoietic cell specific Lyn substrate 1 (general immune) |
| X91144_s_at | PSGL-1 | *Selpl*: selectin, platelet (p-selectin) ligand (general immune) |
| X93037_f_at | WDNM-1 | *Expi*: extracellular proteinase inhibitor (other) |
| X93328_s_at | F4/80 | *Emr1*: EGF-like module containing, mucin-like, hormone receptor-like sequence 1 (macro/APC) |
| Y00635_s_at | CD3 | *Cd3g*: CD3 antigen, γ polypeptide (T cell) |
| Z12297_s_at | MCP-3 | *Scya7*: small inducible cytokine A7 (general immune) |
| X97227 | CD53 | *Cd53*: CD53 antigen (macro/APC) |
| X16151 | Eta-1/osteopontin | *Spp1*: secreted phosphoprotein 1 (T cell) |
| U21795 | Il2rg | IL-2 receptor, γ chain (T cell) |
| L11613 | LMP2 proteosome | *Psmb9*: proteosome (prosome, macropain) subunit, β type 9 (large multifunctionalprotease 2) (macro/APC) |
| M90397 | bcl-3 | *Bcl3*: B cell leukemia/lymphoma 3 (general immune) |
| M63630 | IRG-47 (ifn-γ regulated GTP binding) | *Ifi47*: interferon γ inducible protein, 47 kDa (T cell) |
| J00475 | IgH chain DJC region | *Igh-2*: immunoglobulin heavy chain 2 (serum IgA) (general immune) |
| U60087 | TAP2 | *Abcb3*: ATP-binding cassette, subfamily B (MDR/TAP), member 3 (macro/APC) |
| V00832 | MHC-II α | *H2-Aa*: histocompatibility 2, class II antigen A, α (k haplotype) (macro/APC) |
| K01923 | MHC-II-Ia-α | *H2-Aa*: histocompatibility 2, class II antigen A, α (d haplotype) (macro/APC) |
| M86390 | Moesin | *Msn*: moesin (other) |
| U22031 | Large multiprotease 7 | *Psmb8*: proteosome (prosome, macropain) subunit, β type 8 (large multifunctional protease 7) (macro)/APC |
| U34277 | PAF acetylhydrolase | *Pla2g7*: phospholipase A2 group VII (platelet-activating factor acetylhydrolase, plasma) (general immune) |
| Y07836 | Basic–helix–loop–helix | *Stra14*: stimulated by retinoic acid 14 (other) |
| X12905 | Properdin | *Pfc*: properdin factor, complement (general immune) |
| L20315 | Mpeg1 | Macrophage expressed gene 1 (macro) |
| X00619 | Tcrb-V13 | T cell receptor β, variable chain 13 (T cell) |

signal of 6-carboxyfluorescein (6-FAM) during the amplification cycle. Quantitation of gene-specific message levels was based on a comparison of the fluorescent intensity in the unknown mRNA sample with the fluorescence intensity from a standard curve of known mRNA levels. Amplification of mouse glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) cDNA was performed on all samples tested to control for variations in amounts of RNA. Levels of gene-specific messages were graphed as normalized message units as determined from the standard curve (Kruse *et al*, 1997; Trepicchio *et al*, 1999). A no template control was included in each amplification reaction to control for contaminating templates. For valid sample analysis the fluorescence intensity in the no template control was required to be zero.

**Cell isolation and transfer** Conditioned spleen cells and lymph node cells from spontaneous AA-affected mice were each examined for their potential to promote AA. AA-affected and normal haired C3H/HeJ donor mice were euthanized. Spleens and lymph nodes (axillary, brachial, superficial cervical, inguinal) were passaged through sterile Nitex 110 mesh (Tetko, Lancaster, NY) with Hank's balanced salt solution (Gibco, Grand Island, NY) on ice. Erythrocytes were removed from spleen cell populations using hemolytic ACK buffer (8.92 g per l $NH_4Cl$, 1 g per l $KHCO_3$, 0.32 g per l $Na_2$ ethylenediamine tetraacetic acid (all Sigma, St Louis, MO) in distilled $H_2O$ and autoclaved) for 10 min at room temperature. Nucleated cells were washed by centrifugation at $200 \times g$ for 5 min at 4°C and resuspended in fresh Hank's balanced salt solution. After determining viable cell concentration by exclusion of 0.1% trypan blue (Sigma), cell samples were resuspended at $5 \times 10^7$ cells per ml.

**Cell transfer to immunocompetent C3H/HeJ mice** Preliminary pilot studies suggested AA could be transferred to unaffected C3H/HeJ mice by a single intraperitoneal injection of $10^7$ lymph node or spleen derived cells from AA-affected mice; however, overt hair loss typically did not develop until 14 wk after injection (data not shown). In this study, 14 mice with spontaneous AA and aged between 6 and 12 mo were used as a source of spleen and lymph node cells. Of 50 recipients

aged 15 wk, 10 females and five males received spleen cells subcutaneously (sc) and 10 females and five males received lymph node cells sc. Ten mice received lymph node cells sc and 10 mice received spleen cells sc from normal haired donor mice, age range 4–6 mo. All mice received $10^7$ cells in 0.2 ml of Hank's balanced salt solution.

***In vivo* CD8$^+$ or CD4$^+$ cell depletion in spontaneous AA-affected C3H/HeJ mice** All monoclonal antibodies (MoAb) were produced by the Jackson Laboratory Monoclonal Antibody Service from publicly available hybridoma cell clones (American Type Culture Association, ATCC, Rockville, MD). Rat IgG2b isotype MoAb from clones 53–6.72 and GK1.5 target and deplete mouse CD8$^+$ or CD4$^+$ cells, respectively. Purified rat IgG (Dako, Carpinteria, CA) was used as a negative control. Of 21 spontaneous AA-affected mice with an age range of 5–10 mo, seven received 53–6.72 MoAb, seven received GK1.5 MoAb, and seven received rat IgG. Each mouse received 100 μg of antibody per injection on days 0, 3, 7, and 10 in 0.2 ml of phosphate-buffered saline (PBS) injected intraperitoneally.

Blood samples (≈ 100 μl) from each mouse were taken by retro-orbital bleeding on day 0, immediately prior to the first antibody injection, and subsequently on day 22. Erythrocytes were lysed with hemolytic ACK buffer and leukocytes washed. Each blood sample was aliquoted and incubated with saturating concentrations of fluorescent isothiocyanate conjugated MoAb YTS191 anti-CD4$^+$ MoAb or YTS169 anti-CD8$^+$ MoAb. Each MoAb demonstrates noncompetitive binding with MoAb GK1.5 or 53–6.72. Samples were also simultaneously incubated with phycoerythrin conjugated H57-597 anti-α/β T cell receptor MoAb. The cell samples were washed and a fluorescence-activated cell sorter flow cytometer (Becton Dickinson, Sunnyvale, CA) was used to analyze lymphocyte cell populations using dual color fluorescence analysis. Events were analyzed using appropriate forward and side scatter settings and gates defining the lymphocyte population. Cell viability was assessed by propidium iodide exclusion. Cell debris and dead cells were excluded from analysis.

Mice in these studies were monitored from day 0 at the time of injection onwards by regular examination and macrophotographs taken to record changes. Mice injected with cells were euthanized after onset of AA or after 140+ d without apparent phenotypic change. Cell-depleted mice were euthanized after 50 d. Dorsal and ventral skin were excised for routine histology at the time of necropsy.

**MoAb blockade of lymphocyte activation** MoAb directed against the antigen-presenting cell (APC) surface markers B7.1 (CD80; rat IgG2a clone 1G10.F9) and B7.2 (CD86; rat IgG2a clone GL–1, Wyeth Research, Cambridge, MA) (100 μg per mouse) were injected intraperitoneally 1 d prior to surgery into graft donor mice (those with and without AA) and future graft recipient mice. Following surgery, both antibodies were injected at this concentration three times per week for 4 wk into five female mice that received grafts from female mice with AA and another five females that received grafts from normal female mice all of the C3H/HeJ inbred strain. PBS was injected as a negative control into a second group of five female mice that received grafts from female mice with AA and another five females that received grafts from normal female C3H/HeJ mice. In addition, four C3H/HeJ mice with naturally occurring AA and four that were clinically normal were injected with these MoAb for a period of 4 wk. Mice were observed daily and hair regrowth or alopecia were recorded weekly.

Fifteen mice that received AA skin grafts and 15 that received normal skin grafts were all injected intraperitoneally with 100 μg of mCTLA-4-mIgG2am per mouse (Wyeth Research, Cambridge, MA) (Steurer *et al*, 1995; Ling *et al*, 1998). All mice were injected 1 d prior to receiving the full thickness graft and then three times a week for 1 wk after surgery. A third group of 15 mice received skin grafts from AA mice and were treated the same except they received antibodies for 4 wk after surgery. Mice were observed as described above. Groups of five mice from both those receiving AA or sham grafts were necropsied at 8, 16, and 20 wk after receiving grafts.

## RESULTS

**Histology** Full thickness skin grafts from spontaneous, chronic, AA-affected mice were surgically implanted onto unaffected, syngeneic littermates. Only mice with grafts that exhibited no signs of hair loss due to technical complications were used in this study. To determine histologic and molecular events in advance of overt hair loss, total body skin was harvested from mice at 2 wk intervals after the time of grafting. Because hair loss can begin at distant locations as well as sites adjacent to the graft, the entire body skin was removed and used from these mice. Skin distant from the graft sites with anagen stage hair follicles had perifollicular and intrafollicular lymphocytic infiltrates typical of the C3H/HeJ mouse AA 8 wk after engraftment (**Fig 1**). No changes were observed in sham-grafted mice (**Fig 1**). Detailed histologic and cell typing studies are presented elsewhere (Zoller *et al*, 2002).

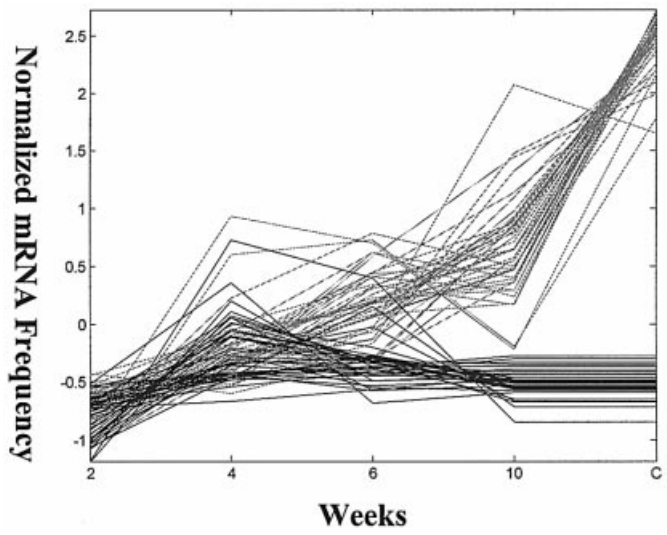


**Figure 2. Clustering of genes elevated in mice receiving alopecia areata affected skin grafts.** Normalized mRNA frequency (a statistical transformation of absolute mRNA frequency which allows clustering independent of expression magnitude) is plotted against weeks after grafting or in chronic (C) cases (mice with spontaneous, long-standing AA or age- and sex-matched normal mice). Black lines are data from mice receiving normal skin grafts (controls) or age-matched mice receiving normal skin grafts. Grey lines are results from mice that received AA skin grafts or those with chronic disease. The genes populating this cluster are listed in **Table I**.

**Gene array analysis of induced mouse AA** In order to understand the molecular kinetic events in the pathogenesis of AA, RNA was isolated from total skin at the various time points from AA-grafted and control-sham grafted mice as well as naturally affected C3H/HeJ mice with long-standing AA. Analyses using the SubB chip of the Affymetrix mouse 11K® chip were performed on RNA obtained from skin collected at 2, 4, 6, and 10 wk after surgery as well as from spontaneous, long-standing (chronic) AA-affected mice. Cluster analysis of the data using a self-organizing map algorithm showed unique classes of genes that were changed at different time points (Wilson *et al*, 2000). Only transcript changes ⩾ 3-fold were considered significant, and one of the genes had to be "present" in at least one of the samples (parameters defined by Affymetrix Gene chip software). **Table I** and **Figs 2** and **3** show transcripts that were elevated early (4–6 wk after grafting) in the skin-grafted AA-affected mice. Of 42 known genes present in this cluster, transcription of T cell marker genes such as *Cd3*, *Cd48*, *Cd53*, and *Selpl* was elevated, as were macrophage/APC markers *H2*, *Emr1*, *Psmb9*, and *Abcb3*. These expression patterns are consistent with early immune infiltrates into the grafts consisting of both T cell and macrophages/APC. At later time points after grafting, numerous transcripts encoding a variety of antibody-variable chain transcripts were observed, suggesting a global humoral or polyclonal B cell activation response present in these mice late in the pathogenesis of AA (> 10 wk, **Table II**). Collectively, these data provide an overview of the molecular immune progression of AA in mice, beginning with early markers of the immune response involving tissue inflammation and vasodilatation, proceeding to macrophage/APC activation and T cell activation, and culminating in the production of transcripts associated with antibody production.

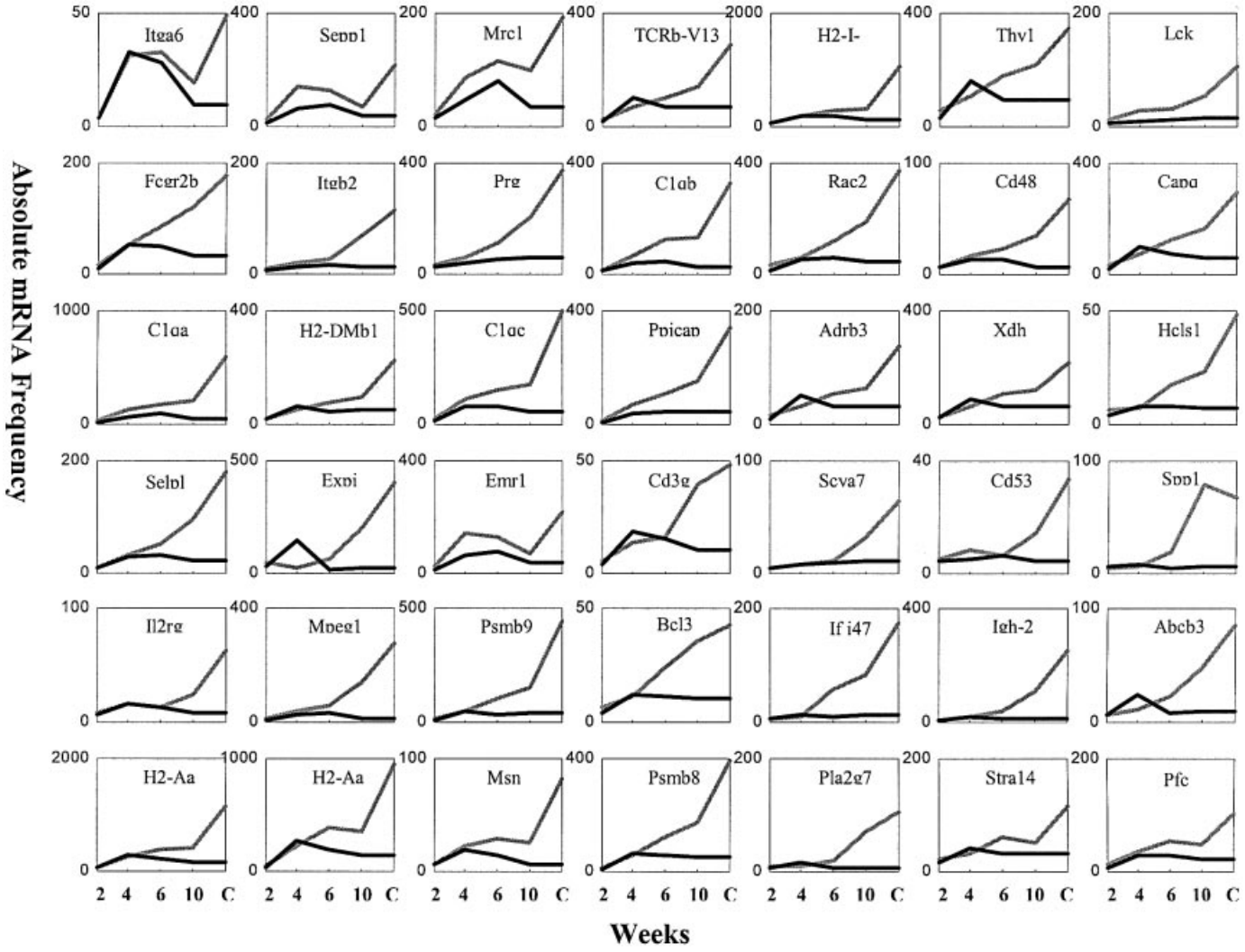


**Figure 3. Each gene listed in Table I and summarized in Fig 2 is plotted individually using absolute mRNA frequency to illustrate the relative strength of each signal.** Black lines are data from control grafted mice, whereas grey represent AA grafted mice.

The normal pathway of T cell activation requires two main signals (Bugeon and Dallman, 2000; Salomon and Bluestone, 2001). Signal 1 is achieved through an interaction of the T cell receptor with antigen in the context of H2 (mouse histocompatibility locus)/HLA (human leukocyte antigen locus) complex molecules, whereas Signal 2 is via CD28 on the T cell surface interacting with B7 family members on the APC surface. Transcripts for costimulation genes were slightly elevated. Quantitative PCR analysis was therefore done to confirm this observation using the same mouse skin used for microarray analyses to ascertain expression level changes in the molecules associated with T cell activation (Salomon and Bluestone, 2001). As shown in **Fig 4(*A,B*)**, transcripts for both B7.1/B7.2 and CTLA-4 are significantly elevated by 6 wk and 4 wk, respectively, consistent with the microarray data and the concept that macrophage activation is coincident or occurs just prior to T cell activation. Quantitative PCR analysis of other costimulatory pathways was also done. *PD-1* (programmed cell death 1) and *ICOS* (inducible T cell costimulator) transcripts were highly elevated during early disease (**Fig 4*B***). These data show that transcripts for various costimulation pathways were present in the skin of these mice, suggesting their casual involvement in the phenotype. We also wanted to determine if the T cell immune infiltrate was associated with a T helper (Th)1 or Th2 cytokine profile. As shown in **Fig 4*C***, early induction of interferon-γ (*Ifn-γ*) is observed, whereas no heightened expression of interleukin 4 (*Il-4*) was seen. This profile is consistent with a Th1 immune infiltrate in the skin of AA mice.

**Gene array analysis of chronic human AA** To compare human AA with C3H/HeJ mouse AA, RNA from three patients and four unaffected age-, sex-, and biopsy site-matched people was examined using Affymetrix human 6800® chips. Samples were run individually. The mean frequencies were grouped and analyzed statistically. Genes that changed $<$ or $>$ 2-fold, with a $p < 0.05$ between the control and AA patients, are listed in **Tables III** and **IV**.

Biopsies were taken from human patients with chronic, nonresponsive AA, i.e., those with no active inflammation and relatively few hair follicles/biopsy. Yet, significant immune changes were observed. In the group of genes that were upregulated in human AA patients compared with controls, 31 genes fit the exclusion criterion used (**Table III**). Of these, 11 are associated with lymphocytic infiltrates. Many genes were suggestive of innate immunity, such as mast cell markers (*Il-8rb*, chemokine (C-X-C) receptor 2, *Cmkar2*) and the chemokine *Mcpt4* (mast cell protease 4), associated with allergic disease, were elevated. Immune markers associated with T cell infiltrates were also observed. Granzyme A, expressed in $CD4^+$ T cells, was elevated in the skin, as was *NFATC3* (nuclear factor for activated T cells, cytoplasmic 3), a

**Table II. Upregulated transcripts (≥ 3-fold) of genes coding for immunoglobulin variable chains in mice receiving AA-affected skin grafts 10 wk after surgery**

| GenBank accession no.[a] | Transcript |
|---|---|
| ET61657 | *Mus musculus* clone 11E6 IgG anti-nucleosome κ light chain |
| ET61730 | *Mus musculus* clone immunoglobulin 2G3.H5 heavy chain mRNA specific for mouse cytochrome c |
| ET61785 | *Mus musculus* anti-DNA immunoglobulin heavy chain IgM antibody 363p.168 |
| ET61855 | *Mus musculus* anti-DNA immunoglobulin heavy chain IgG antibody 423p.135 |
| ET61919 | *Mus musculus* anti-DNA immunoglobulin heavy chain IgM antibody 363s57 |
| ET61989 | *Mus musculus* anti-DNA immunoglobulin heavy chain IgG antibody 423s17 |
| ET62199 | *Mus musculus* immunoglobulin anti-DNA light chain (Vk4/5) |
| ET62234 | *Mus musculus* polyreactive autoantibody, immunoglobulin IgM heavy chain mRNA |
| ET62762 | *Mus musculus* anti-von Willebrand factor antibody NMC-4 κ light chain mRNA |
| ET62868 | *Mus musculus* anti-CD8 immunoglobulin heavy chain V region mRNA |
| ET62983 | *Mus musculus* mRNA for (2F7) IgA V–D–J region |
| ET63126 | *Mus musculus* mRNA for anti-folate binding protein, MOv19 Vκ |
| ET63276 | *Mus domesticus* IgK variable region (PIR:S26746 immunoglobulin heavy chain J regionJH3-mouse) |

| GenBank accession no. | Common gene name | Official gene symbol: name |
|---|---|---|
| U22519 | Angiogenin-related protein (Angrp) | *Angrp*: angiogenin-related protein |
| L43568 | B cell receptor gene | *Igh-J*: immunoglobulin heavy chain, joining region<br>*Igh-VJ558*: immunoglobulin heavy chain (J558 family)<br>*Igk-J*: immunoglobulin κ chain, joining region<br>*Igk-V5*: immunoglobulin κ chain variable 5 (V5 family) |
| X54424 | γ adaptin | *Ap1g1*: adaptor protein complex AP-1, γ1 subunit |
| X88903 | *Mus musculus* mRNA for variable light | *Igk-V38*: immunoglobulin κ chain variable 38(V38) chain |
| M80423 | *Mus musculus castaneous*. IgK chain gene, | *Igk-C*: immunoglobulin κ chain, C-region, 3′ end constant region |
| x56602 | *Ifn*-induced 15 kDda protein | *Isg15*: interferon-stimulated protein (15 kDa) |
| x67783 | VCAM-1 | *Vcam1*: vascular cell adhesion molecule 1 |
| x85999 | IL-1 accessory protein | *Il1rap*: IL-1 receptor accessory protein |
| x62742 | – | *H2-DMa*: histocompatibility 2, class II, locus Dma |

[a]Twenty-six additional Affymetrix clones represent different clones for the same antibodies list above. Of 114 known genes, 48 represent transcripts to antibodies.

signaling molecule associated with T cells. Increased *STAT1* (signal transducer and activator of transcription 1) expression is indicative of Ifn-γ signaling as this transcript is often upregulated in Ifn-γ responsive cell types. Interleukin-1 receptor (*Il1r1*) was also upregulated, consistent with previous observations of this pathway playing a part in this disease (Tarlow *et al*, 1994). In addition, an array of metabolic, adhesion, and signaling molecules were also upregulated in these biopsies. Previous association of these transcripts with AA has not been observed.

The exclusion criteria used defined 64 downregulated genes (**Table IV**). Of these, 15 are associated with hair keratins or hair follicles. Many of these transcripts are decreased to the point of being absent. The remaining downregulated transcripts were generally associated with either hair follicle or epidermal differentiation and may reflect a molecular fingerprint of the transcripts' status in the skin of patients. Not all transcripts specifically associated with hair follicles were downregulated. Transcripts for trichohyalin were unchanged between control and AA biopsies, suggesting that not all hair follicle associated transcripts are downregulated in chronic, dystrophic human AA hair follicles, as hair fibers continue to be produced in AA patients. Hair keratins were also downregulated in mice with long-standing AA (**Table V**).

**Cell transfer to immunocompetent C3H/HeJ mice** Subcutaneous injection of lymph node cells from AA-affected mice to normal, haired, immunocompetent C3H/HeJ mice was an effective promoter of AA. Ten females and two males of 15 mice (80%) developed ventral and/or dorsal patchy AA during the study. Hair loss was first observed in eight mice by 5 wk at the immediate site of cell injection. One mouse had hair loss at the injection site for the duration of the study but did not develop further hair loss. Of 11 mice with systemic hair loss (73%), onset distant to the injection site was first observed 7–10 wk after cell administration (**Fig 5**). Hair loss persisted until the time of necropsy. By contrast, five females and one male of 15 mice (40%) developed AA with first onset 7–10 wk after sc injection of spleen cells. sc injection of spleen cells was not associated with hair loss localized at the site of injection. No mice injected with spleen or lymph node cells derived from normal haired mice developed AA during the course of the study.

The histopathology of mice with hair loss revealed perifollicular and intrafollicular infiltrates of inflammatory cells, primarily lymphocytes, typical of those observed in C3H/HeJ mice with spontaneous AA (Sundberg *et al*, 1994a; Freyschmidt-Paul *et al*, 1999). Various degrees of infiltration were observed in different mice and at different biopsy sites from each mouse as is typical of the spontaneous disease. Inflammation was primarily composed of mononuclear cells. Those with no hair loss had no apparent anagen hair follicle dystrophy or inflammation.

***In vivo* CD8$^+$ or CD4$^+$ cell depletion in spontaneous AA-affected C3H/HeJ mice** Flow cytometry demonstrated successful depletion of CD4$^+$ or CD8$^+$ cells from peripheral blood samples of respective experimental mice (**Fig 6**, **Table VI**). No further immune manipulation was conducted on the cell-depleted mice. Mice were permitted to regenerate their CD4$^+$ and CD8$^+$ cell numbers. Of seven mice depleted of CD4$^+$ cells, three showed significant hair regrowth with the first observation for one mouse on day 27. Two showed limited, sparse hair regrowth, and two showed no apparent regrowth of hair. All mice responding to CD4$^+$ cell depletion showed hair regrowth by 34 d after initiation of the study. Pelage regeneration reached a maximum by day 41, although the hair coat was not complete in any mouse (**Fig 7**).

For seven mice depleted of CD8$^+$ cells, significant hair regeneration was noted in one and sparse hair growth in three. Three mice showed no apparent regrowth response. First hair regrowth was noted by day 31 and all responding mice showed regrowth by day 41. Of seven control mice injected with rat IgG, one showed sparse hair regrowth, two showed no apparent

regrowth, and four had progressive hair loss. These results suggest that both $CD4^+$ and $CD8^+$ cells are involved in AA.

**Intervention with MoAb** In order to ascertain whether the upregulation of costimulatory and T cell activation molecules was involved causally in the onset of AA in these mice, reagents known to diminish T cell activation by preventing B7–CD28 interactions were used. Full thickness skin grafts from C3H/HeJ mice with AA to clinically normal C3H/HeJ age- and sex-matched mice reproducibly resulted in AA in 35 graft recipients. Results are summarized in **Table VII**. Treatment of mice with spontaneous AA with MoAb directed against both B7.1 and B7.2 had no effect on the clinical disease in four mice. There was no disease induction in control mice (mice that received normal skin grafts and were treated with either mCTLA-4-mIgG2am ($\chi^2 = 1.185$, $p = 0.276$) or B7 (NS) there were no significant differences. There was no significant difference in the treated or untreated mice with or without (AA). Treatment before the onset of AA, however, prevented disease in all five mice indicating that the B7 blockade affects disease (anti-B7 *vs* PBS results were significantly different ($\chi^2 = 9.0$, $p = 0.0027$) onset but not active lesions. Isotype controls responded similarly to PBS in previous studies (Freyschmidt-Paul *et al*, 2000b). Therefore, B7 blockade does not reverse the hair-loss phenotype. Similarly, mCTLA-4-mIgG2am competed effectively with CD28 for B7.2, preventing interaction with CD28 and thereby preventing onset of AA in 13 of 15 and 14 of 15 mice receiving AA grafts when administered for 1 or 4 wk, respectively. As there were no statistical differences between mCTLA-4-mIgG2am used for 1 or 4 wk, these data were pooled. For mice that received AA skin grafts no treatment was significantly different from those getting mCTLA-4-mIgG2am ($\chi^2 = 53.9$, $p = 0.0001$).

## DISCUSSION

AA can be induced in C3H/HeJ mice by transferring full thickness alopecic skin grafts from a spontaneously AA-affected mouse to normal haired, immunocompetent mice (McElwee *et al*, 1998a). Initial studies focused on refining the criteria needed to generate a reproducible model (McElwee *et al*, 1998a). More recently, this mouse model was used to test compounds that are efficacious on human patients and for determining efficacy of MoAb that block lymphocyte migration and prevent AA (Freyschmidt-Paul *et al*, 1999, 2000a, b, 2001; Gardner *et al*, 2000).

The objective of this study was to investigate mechanisms of disease progression leading to clinical onset and to compare these results with the spontaneous, chronic form of AA found in the C3H/HeJ inbred mouse strain. Furthermore, mouse results were compared with the natural, chronic human forms of AA. Analysis of gene expression changes in the mouse model were compared with those in human patients with the chronic forms of AA, AT, and AU, to determine the degree of similarity between the species.

The kinetic progression of the RNA profile changes in the AA mouse model is consistent with an autoimmune mechanism of hair loss. Downregulation of hair keratins and hair follicle-associated genes at 4–6 wk postgrafting is coincident with the presence of markers of activated macrophages/APC and activated T cells.

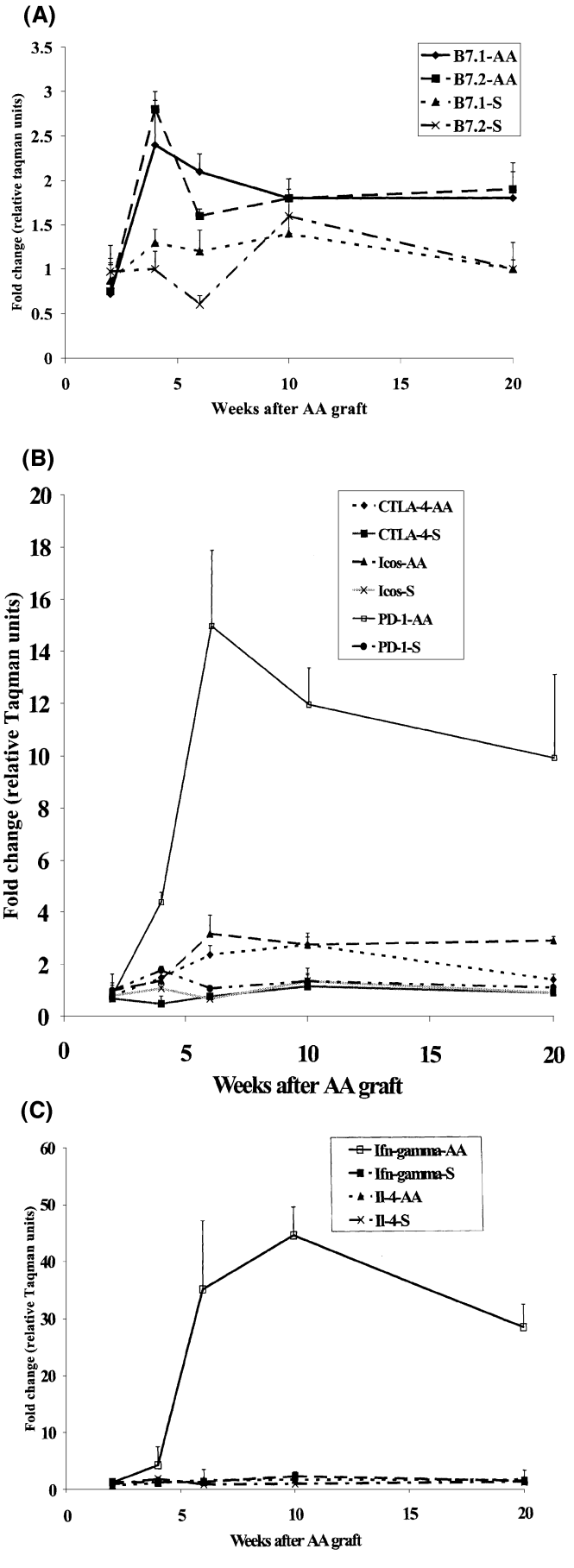


**Figure 4. Increased costimulatory molecule and cytokine expression in alopecia areata development.** (*A*) Quantitative PCR reveals early upregulation of B7.1 and B7.2 transcripts in mice receiving the AA grafts (AA), whereas those receiving normal, unaffected skin (sham grafts, S) exhibited no changes. (*B*) Quantitative PCR revealed an upregulation of $PD^{-1}$ as well as *CTLA-4* and *Icos* transcripts 2 wk after mice received AA affected grafts. No changes were found in these transcripts in mice that received normal, unaffected skin grafts. (*C*) Quantitative PCR demonstrated a marked upregulation of *Ifn-γ* but not *Il-4* transcripts in mice receiving AA affected grafts. No changes were detected for these transcripts in mice receiving normal, unaffected skin grafts.

**Table III. Transcripts upregulated (≥ 2-fold) in human AA patients compared with age-, sex-, and biopsy site-matched control individuals**

| GenBank accession no. | Common gene name | Official gene symbol: name | Fold change |
|---|---|---|---|
| M28826 | Cd1a[a] | *CD1A*: CD1A antigen, a polypeptide | 2.4 |
| L00389 | Cyto-P450 | *CYP1A2*: cytochrome P450, subfamily I (aromatic compound-inducible), polypeptide 2 | 3.4 |
| M77140 | Galanin[a] | *GAL*: galanin | 7.1 |
| M18737 | Granzyme A | *GZMA*: granzyme A (granzyme 1, cytotoxic T lymphocyte-associated serine esterase 3) | 2.6 |
| U21936 | Pept-1 | *SLC15A1*: solute carrier family 15 (oligopeptide transporter), member 1 | 2.7 |
| J05582 | Muc-1 | *MUCI*: mucin 1, transmembrane | 2.3 |
| L41067 | NFAT C3[a] | *NFATC3*: nuclear factor of activated T cells, cytoplasmic, calcineurin-dependent 3 | 2.3 |
| U46767 | MCP-4 | *SCYA13*: small inducible cytokine subfamily A (Cys-Cys), member 13 | 3.1 |
| D16294 | Mitochondrial coenzyme A thiolase | *ACAA2*: acetyl-coenzyme A acyl-transferase 2 (mitochondrial 3-oxo-acyl-coenzyme A thiolase) | 6.2 |
| L19593 | IL-8R[a] | *IL8RB*: IL-8 receptor, β | 3.6 |
| U02493 | FKBP5 | *FKBP5*: FK506-binding protein 5 | 4.2 |
| M87770 | FGFR2 | *FGFR2*: fibroblast growth factor receptor 2 | 3.7 |
| X97324 | Adipophilin[a] | *ADFP*: adipose differentiation-related protein | 5.4 |
| L36529 | STAT-1 | *STAT1*: signal transducer and activator of transcription 1, 91 kDa | 2.9 |
| X59770 | IL-1R2 | *IL1R2*: IL-1 receptor, type II | 2.5 |
| U32324 | IL-11Ra | *IL11RA*: IL-11 receptor, α | 3.4 |
| X67325 | ISG 12 (ifn-γ induced)[a] | *IFI27*: interferon, α-inducible protein 27 | 5.8 |

[a]Not present in controls.

**Table IV. Transcripts downregulated (≥ 2-fold) in human AA patients compared with age-, sex-, and biopsy site-matched control individuals**

| GenBank accession no. | Common gene name | Official gene symbol: name | Fold change |
|---|---|---|---|
| X86570 | Hair acidic keratin 1 (Ha1) | *KRTHA1*: keratin, hair, acidic,1 | – 50 |
| X82634 | Hair acidic keratin 3-II (Ha3b) | *KRTHA3B*: keratin, hair, acidic,3B | – 45 |
| X90763 | Hair acidic keratin 5 (Ha5) | *KRTHA5*: keratin, hair, acidic, 5 | – 13 |
| X81420 | Hair basic keratin 1 (Hb1) | *KRTHB1*: keratin, hair, basic, 1 | – 28 |
| X99141 | Hb3 | *KRTHB3*: keratin, hair, basic, 3 | – 17 |
| X99140 | Hb5 | *KRTHB5*: keratin, hair, basic, 5 | – 32 |
| Z19574 | K17 | *KRT17*: keratin 17 | – 5 |
| X12876 | K18 | *KRT18*: keratin 18 | – 4.3 |
| X07696 | High-sulfur keratin 1 | *KRT15*: keratin 15 | – 7.3 |
| U42408 | Ladinin | *LAD1*: ladinin 1 | – 2.8 |
| M13903 | Involucrin | *IVL*: involucrin | – 3.4 |
| Z34974 | Plakophilin | *PKP1*: plakophilin 1 | – 3.3 |
| M19888 | SPR1 | *SPRR1B*: small proline-rich protein 1B (cornifin) | – 5.8 |
| M20030 | SPR2 | *SPRR2C*: small proline-rich protein 2A | – 7.1 |
| L32137 | COMP | *COMP*: cartilage oligomeric matrix protein | – 6.8 |

**Table V. Downregulated transcripts (≥ 3-fold) in mice that received AA affected skin grafts 10 wk after surgery and in mice with spontaneous, long-standing (chronic) AA (seven known genes, five expressed sequence tags)**

| GenBank accession no. | Common gene name | Official gene symbol: name |
|---|---|---|
| U78109 | Preproneurturin | *Nrtn*: neurturin |
| W45778 | vWF | *Vwf*: Von Willebrand factor homolog |
| X85990 | Semaphorin A (semaphorin) 3B | *Sema3b*: sema domain, immunoglobulin domain, short basic domain, secreted |
| AA108571 | sim to synuclein | *Sncg*: synuclein, γ |
| M23383 | Glucose transporter 2 | *Slc2a4*: solute carrier family 2 (facilitated glucose transporter), member 4 |
| M92088 | Type II hair keratin—Hb6 | *Krt2–10*: keratin complex 2, basic, gene 10 |
| M73483 | Glutathione-S-transferase | *Gsta3*: glutathione-S-transferase, α3 |

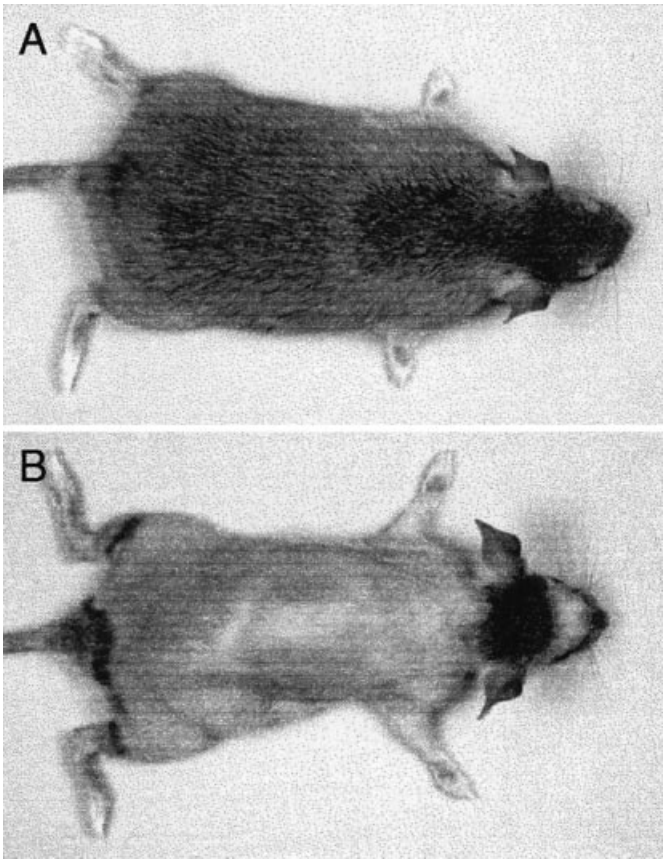


**Figure 5. Transfer of alopecia area with injection of lymph node cells.** Normal haired C3H/HeJ recipient (*A*) of lymph node cells. Ten weeks later this mouse developed extensive alopecia over the head, dorsal thoracolumbar region (*B*), and entire ventral surface (not shown).

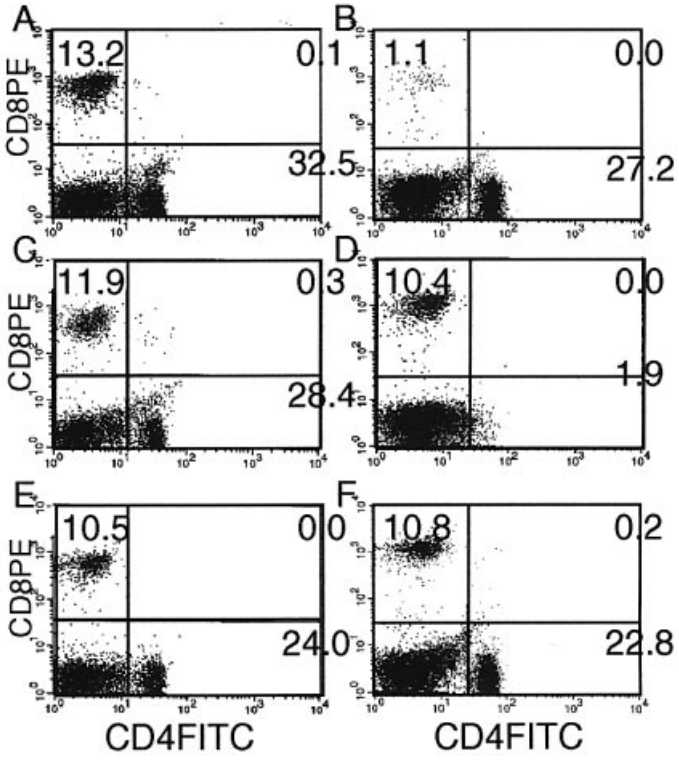


**Figure 6. Fluorescence activated cell sorter analyses confirm cell depletion.** Analysis of peripheral blood from three separate mice prior to injection (day 0; *A*, *C*, *E*) and 22 d after injection (*B*, *D*, *F*) with MoAb. These antibodies selectively depleted $CD8^+$ (*B*) or $CD4^+$ (*D*) lymphocytes, whereas normal rat immunoglobulin had no effect (*F*).

These data suggest that the likely mechanism of hair loss in the mice is initiated by hair follicle-localized antigens being presented to T cells via activated macrophages. As a small skin graft from an affected mouse can initiate this entire autoimmune process, it is possible that the source of immune activation from the graft is either activated APC, memory T cells, or some antigens present in the graft itself.

In the above experiments, both spleen and lymph node cells were capable of promoting AA in C3H/HeJ mice. The delay between subcutaneous cell injection and systemic alopecia was similar to that observed with the skin graft transfer of AA (McElwee *et al*, 1998a). Transfer of skin-draining lymph node cells was more effective than spleen cells in initiating hair loss, suggesting that activated APC or memory T cells from the skin-draining lymph nodes are the primary cells initiating this entire process. Also, RNA analyzed from biopsies of human AA patients in this study showed increased expression of the CD1a Langerhans cell specific marker, suggesting that an ongoing immune response involving APC may also be involved in human AA.

These data are consistent with earlier studies where blockade of T cell migration using an anti-CD44v10 MoAb prevented the onset of AA in the mouse graft model (Freyschmidt-Paul *et al*, 2000b). Confirming the crucial role of T cell activation via APC costimulation, blockade of CD28–B7 interactions and mCTLA-4-mIgG2am competition with CD28 delayed AA onset. In both of these systems, histologic analysis revealed hair follicles without typical lymphocytic infiltrates. The latter approach was used to promote islet graft tolerance in mice (Steurer *et al*, 1995). *In vivo* cell depletion in C3H/HeJ mice resulted in transient hair regrowth or disease stabilization for all C3H/HeJ mice in contrast to control mice where progressive hair loss was predominant. A hair regrowth response with $CD4^+$ or $CD8^+$ cell depletion was demonstrated in a rat AA model (McElwee *et al*, 1996, 1999b). Both $CD4^+$ and $CD8^+$ cells may closely interact to maintain an AA lesion. These observations suggest that disintegration or collapse of hair follicle integrity does not invoke the immune response, rather that the immune system is initiating a hair-follicle specific attack. Consistent with these data, the earliest markers of perturbation of gene expression in the skin of AA mice involve the cell-mediated immune system, not hair follicle associated gene expression changes.

As in AA-affected humans, C3H/HeJ mice with spontaneous or graft-induced AA make transcripts for antibodies directed against many self antigens (Tobin *et al*, 1994, 1997a, b). The probes recognize transcripts previously characterized to produce antibodies against antigens such as DNA (Mohan *et al*, 1998; Sobel *et al*, 1999). Furthermore, the antibody transcripts are directed toward virtually every immunoglobulin isotype. This suggests that the C3H/HeJ mouse has a global propensity towards autoimmunity that is initiated within the hair follicle but then quickly spreads to many self antigens. A similar phenomenon has been observed for antibodies to enteric bacteria associated with the sensitivity of C3H/HeJ mice to inflammatory bowel disease (Sundberg *et al*, 1994b; Brandwein *et al*, 1997). Furthermore, C3H/HeJ mice have a defective response to bacterial lipopolysaccharides (Poltorak *et al*, 1998; Qureshi *et al*, 1999), due to a mutation in the Toll4 receptor, indicating these mice have abnormal innate immunity. This may be true in humans as well, as humans with AA may have other autoimmune diseases such as vitiligo, systemic lupus erythematosus, autoimmune thyroiditis, inflammatory bowel disease, and other diseases (Werth *et al*, 1992; Treem *et al*, 1993; McElwee *et al*, 1999c). Although these data suggest that both the human and the mouse diseases are autoimmune in nature, the primary disease-

**Table VI. Fluorescence-activated cell sorter analyses confirming lymphocyte depletion studies**

| | Day 0 | | Day 22 | |
|---|---|---|---|---|
| MoAb | $CD4^+$ cells | $CD8^+$ cells | $CD4^+$ cells | $CD8^+$ cells |
| Clone Gk 1.5 anti-CD4 | 18.81 ± 5.12% | 8.49 ± 2.16% | 1.81 ± 0.89% | 15.63 ± 4.42% |
| Clone 53–6.72 anti-CD8 | 22.01 ± 6.59% | 9.57 ± 1.69% | 28.06 ± 4.06% | 0.58 ± 0.26% |
| Rat IgT Control | 18.85 ± 4.13% | 9.97 ± 1.33% | 21.31 ± 3.59% | 11.28 ± 2.38% |

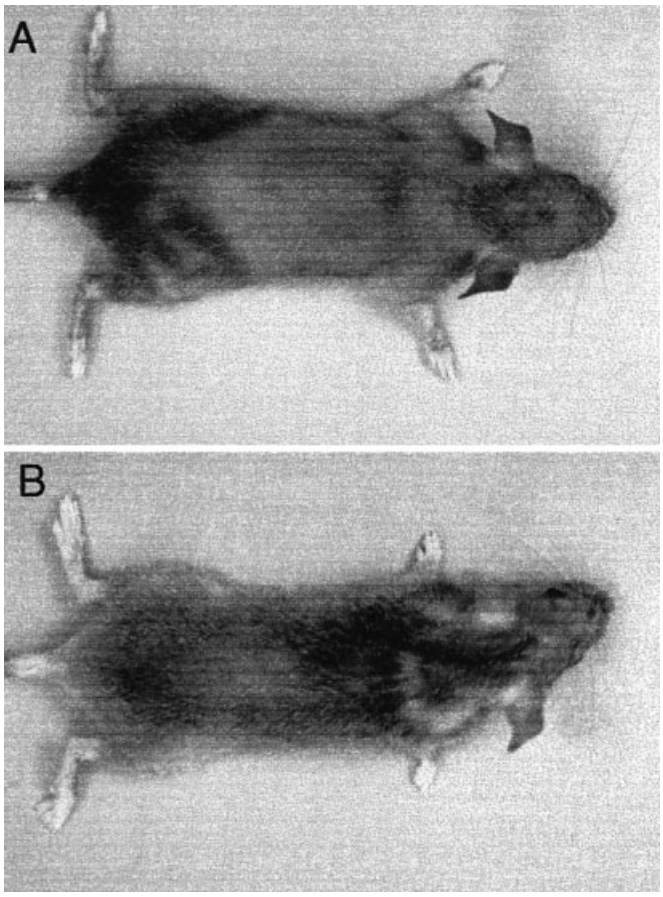


**Figure 7. Hair regrowth occurred after depletion of $CD4^+$ lymphocytes.** Prior to injection of antibodies, the 7 mo old C3H/HeJ mouse exhibited extensive dorsal and ventral alopecia (*A*). After depletion of $CD4^+$ lymphocytes for 50 d, there was hair regrowth with an improved pelage on both dorsal and ventral surfaces (*B*).

**Table VII. Summary of the number of mice that developed AA over those in the test group (*) in response to MoAb treatment**

| | | CTLA-4 | | | |
|---|---|---|---|---|---|
| Graft type | Untreated | 1 wk | 4 wk | Anti-B7 | PBS |
| AA skin graft to recipient | 35/35* | 2/15 | 1/15 | 0/5 | 4/4 |
| Normal skin to recipient | 0/35 | 0/15 | 1/15 | 0/5 | 0/5 |
| Spontaneous AA | 4/4 | NT | NT | 4/4 | NT |
| Normal C3H mouse | 0/4 | NT | NT | 0/4 | NT |

NT, not tested.

activating target remains elusive. Consequently, a conclusive statement on the nature of AA pathogenesis cannot be made.

Expression profiling of human AA biopsies revealed the presence of changes associated with chronic, innate immunity, including expression of surface markers for mast cells as well as the chemokine *MCP4*. Although it is difficult to obtain human hair follicle biopsies in early stages of AA and impossible to obtain biopsies in advance of overt hair loss onset, the mouse data suggest that the initiation phase of this disease phenotype is a heavily Th1-biased immune response. Some genes upregulated in human biopsies are consistent with an immune infiltrate containing Ifn-γ driven, Th1 T cells (*NFATC3, STAT1a*). High levels of *Ifn-γ* and *Ifn-γ*-associated transcripts were detected confirming previous observations about the expression of Th1-related genes in human biopsies (Hoffmann *et al*, 1994). Genes associated with more generalized, innate immune responses were detected in human biopsies (e.g., *Il8rb, Mcpt4*). Many persistent inflammatory diseases initiated via Th1-directed mechanisms become Th2 in the chronic state (HogenEsch *et al*, 2001). This would suggest that maintenance or lack of outright destruction of the hair follicles by cytotoxic cells may be due to a shift from a Th1 response to a more chronic Th2 immune profile. These studies suggest that in the C3H/HeJ mouse model AA is a cell-mediated autoimmune disease with late, possibly secondary, humoral responses. Blockade of parts of the lymphocyte costimulatory cascade prevents onset of AA in the mouse graft-induced model suggesting that similar approaches in human patients will prevent the development of new lesions. By contrast, targeting lymphocytes should control and resolve existing lesions. Gene array derived data have provided a significant insight into AA and the technology is potentially a powerful aid to understanding other chronic inflammatory diseases.

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